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ORIGINAL ARTICLE

Cytotoxic Isoprenoids from *Xanthium strumarium* Linn.

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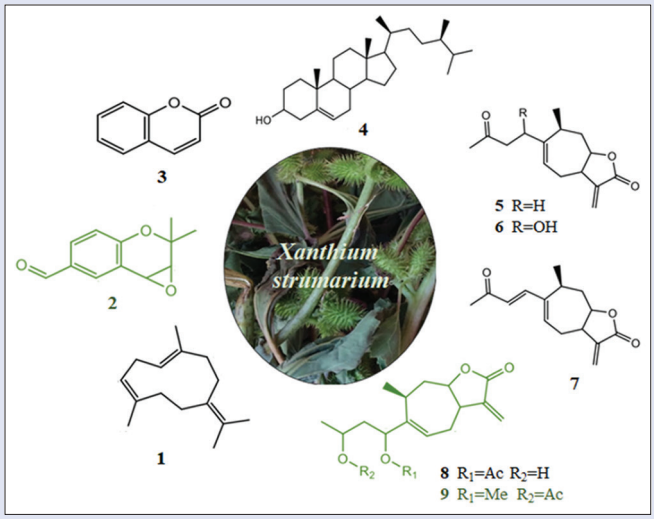
ABSTRACT

Background and Objective: *Xanthium strumarium* is a widespread medicinal plant species; particularly fruits and roots are known for improving memory, voice, and appetite as well as curing of poisonous bites of insects and epilepsy. **Materials and Methods:** The aerial parts of *X. strumarium* were extracted with a combination of organic solvents. The exhaustively dried organic extract was fractionated until obtaining pure individuals by employing the appropriate chromatographic techniques. The spectral information obtained from different nuclear magnetic resonance experiments, mass, infrared, and ultraviolet spectra were the keys to elucidate the chemical structures. **Results:** Nine compounds (1-9) were obtained: a germacrane sesquiterpene (1), five xanthatin-type sesquiterpenoids (5-9) with α-methylene-γ-lactone moiety, including the new one, methoxy xanthanol (9), a benzopyran derivative not previously found in nature 3,4-diepoxy-2,2-dimethyl-2H-1-benzopyran-6-carboxaldehyde (2), and coumarin (3), along with the C-28 steroid campesterol (4). **Conclusion:** Most of the compounds under the study showed an appreciated cytotoxic activity against HCT116 and HepG2 cancer cell lines.

Key words: *Asteraceae*, cytotoxicity, medicinal plants, *Spectroscopy*, terpenoids

SUMMARY

- A new methoxy xanthanol and a new benzopyran derivative in addition to seven known isoprenoids compounds were isolated from *Xanthium strumarium* Linn.
- The biological investigation of most of isolated compounds presented significant activity against HCT116 and HepG2 cancer cell lines.



Abbreviations used: NMR: Nuclear magnetic resonance; MS: Mass spectrometry; UV: Ultraviolet spectroscopy; IR: Infrared radiation; EIMS: Electron ionization mass spectra; TLC: Thin-layer chromatography; PTLC: Preparative thin-layer chromatography; HSQC: Heteronuclear single-quantum correlation; COSY: Correlated spectroscopy; HMBC: Heteronuclear multiple-bond correlation.

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INTRODUCTION

The genus *Xanthium,* with 25 members, is a small genus belonging to the large plant family *Asteraceae*.[1,2] Its species are distributed in almost all continents.[1,2] They were used in the Traditional Chinese Medicine for the treatment of ulcer, arthritis, pruritus, cancer, herpes, and nasal sinusitis.[3-5]

Xanthium strumarium Linn. (Cocklebur) is a herbaceous daisy plant, originated in tropical America and widely distributed in the world.[6] Literature screening of the previous studies focused on the medicinal usage and chemical constituents of *X. strumarium* revealed the broadness appearance in folk medicine as prophylactic, diuretic, sudorific, sialogogue, sedative agent, and a remedy for malaria.[7] It is noteworthy to mention that xantholide, a main constituent sesquiterpene lactone in *X. strumarium*, displayed a high potency against chloroquine-resistant *Plasmodium falciparum*. Polyphenols, plastoquinone, and tocopherols are

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the main constituents of the fruits of *X. strumarium*, whereas the aerial parts showed to contain daucane- and xanthane-type sesquiterpenoids.[7,8]

In pharmaceutical industry, the combinatorial chemistry has attracted the attention to the synthetic rather than the natural compounds due to high-throughput and large-scale applications.[9] Nevertheless, natural compounds are more diverse and supply higher structural complexity than the synthetic equivalents.[9] Moreover, in drug-like enrichment, natural metabolites are much higher than the synthesized counterparts.[10]

Colorectal cancer is already the third leading cause of cancer death in the world and its incidence is steadily rising in developing nations,[11] while liver cancer is the fifth most common cancer, accounting for 9.1% of all cancer deaths worldwide[12] This manuscript is interested in the isolation and evaluation of the cytotoxicity of secondary metabolites from the indigenous medicinal plant *X. strumarium*, known in the Arabian area as "Losiq." It was collected from River Nile State, Sudan. Its organic extract afforded nine compounds 1–9: six sesquiterpenoids 1 and 5–9, a new natural benzopyran derivative (2) previously known as synthetic product, and coumarin (3) along with the C-28 steroid, campesterol (4). All compounds were tested against two cancer cell lines HCT116 (colon cancer) and HepG2 (liver cancer) using sulforhodamine B assay. Most of the compounds under the study showed an appreciated cytotoxic activity against HCT116 and HepG2 cancer cell lines.

MATERIALS AND METHODS

General

The materials and methods employed in this article are detailed elsewhere.[13]

Plant material

X. strumarium was collected from River Nile State, Sudan, in March 2017, and was identified by Prof. Jamal Sabar, Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University (KAU), Jeddah, Saudi Arabia. A voucher specimen of *X. strumarium* has been deposited at the Department of Biological Sciences, Faculty of Sciences, KAU.

Extraction and isolation

The partially air-dried aerial parts (371.0 g) were soaked in a mixture of equal volumes of petroleum ether, methylene chloride, and methanol (4.5 L, two times at room temperature). The extract was then concentrated and successively portioned between *n*-hexane/water and chloroform/water. The chloroform extract (12.42 g) was chromatographed on an aluminum oxide column (750 g, 75 cm × 2.5 cm) and eluted successively with petroleum ether, petroleum ether–ether, and petroleum ether–ethyl acetate. 50 mL fractions were collected, thin-layer chromatography (TLC) was carried out for all fractions, and *p*-anisaldehyde-sulfuric acid and methanol-sulfuric acid reagents were employed for visualization. The fraction eluted with petroleum ether was further purified by preparative thin-layer chromatography (PTLC) (using petroleum ether as eluent); the violet band (R_f 0.76) appeared up spraying with *p*-anisaldehyde-sulfuric acid was collected to give an oily material (1, 1.7 mg). The fraction eluted with 25% diethyl ether in petroleum ether was further purified by PTLC (15% diethyl ether in petroleum ether) to give three bands; the purple band (R_f 0.56) appeared up on spraying with *p*-anisaldehyde-sulfuric acid was collected to give an oily material (2, 0.5 mg). The violet band (R_f 0.29) was collected as colorless solid (3, 1.7 mg). The dark purple band (R_f 0.22) was collected as white solid material (4, 1.4 mg). The fraction eluted with 50% diethyl ether in petroleum ether was further purified by PTLC (50% diethyl ether in petroleum ether) to give a yellowish band (R_f 0.35) which appeared up spraying with *p*-anisaldehyde-sulfuric acid was collected to give an oily material (5, 2.5 mg). The fraction eluted with 30% ethylacetate in *n*-hexane was further purified by PTLC (30% ethylacetate in *n*-hexane) to give a violet band (R_f 0.42) which appeared up spraying with *p*-anisaldehyde-sulfuric acid was collected to give an oily material (6, 1.8 mg). The fraction eluted with 40% ethylacetate in *n*-hexane was further purified by PTLC (50% ethyl acetate in *n*-hexane) to give three bands: a dark violet band (R_f 0.56) appeared up spraying with *p*-anisaldehyde-sulfuric acid was collected to give an oily material (7, 3.5 mg), a reddish band at R_f 0.41 was collected to give an oily material (9, 1.9 mg), and a light violet band at R_f 0.22 was collected to give an oily material (8, 2.0 mg).

Characterization of the isolated compounds

3,4 diepoxy-2,2-dimethyl-2H-1-benzopyran-6- carboxaldehyde (2)

Characterization of the 3,4 diepoxy-2,2-dimethyl-2H-1-benzopyran-6-carboxaldehyde (2) included pale yellow oil (0.5 mg); ultraviolet spectroscopy (UV) λ_{max} (MeOH) 221, 253 nm; IR $\acute{\upsilon}_{max}$ 2925 (CH, st), 1705 (C=O, st), 3054 (=CH, st), and 1680 (C=C, Ar, st), 1497 cm^{-1}; HR-ESI-MS *m/z* 204.0780 $[M]^+$ (Calcd for $C_{12}H_{12}O_3$, 204.0786); ^{1}HNMR (Bruker WM 850 MHz); and ^{13}C Nuclear magnetic resonance (NMR) (212.5 MHz) in $CDCl_3$ [Table 1].[14,15]

Methoxy xanthanol (9)

Characterization of the methoxy xanthanol (9) included colorless oil (2.0 mg); UV λ_{max} (MeOH) 218 nm; IR $\acute{\upsilon}_{max}$ 1375(*gem*-dimethyl, st) 1249(C-O), 1736 (C = O), 1767 (lactone), and 2922(CH) cm^{-1}, HR-ESI-MS *m/z* 322.1774 $[M]^+$(Calcd for $C_{18}H_{26}O_5$, 322.1780); ^{1}HNMR (Bruker WM 850 MHz); and ^{13}C NMR (212.5 MHz) in $CDCl_3$ [Table 1].

Known natural compounds

The further isolated natural metabolites were identified as 1,5-dimethyl-8-(1-methylethylidene)-1,4-cyclodecadiene (1), coumarin (3), campesterol (4), tomentosin (5), 1'-hydroxytomentosin (6), xanthatin (7), and xanthanol (8) after comparison of their spectral and physical properties with the published data.[16-22]

In vitro cytotoxic activity

The detailed biological method was mentioned previously by Skehan *et al.*[23,24]

RESULTS AND DISCUSSION

Chemistry

Nine metabolites (1-9) were the net result of chromatographic separation of the organic extract of *X. strumarium*, out of them, two new natural compounds were identified: 2 (0.5 mg, 0.0001% yield), previously known as a synthetic product,[14,15] and 9 (2 mg, 0.0005% yield).

Compound 2 was isolated as pale yellow oil with specific rotation $[\alpha]_D$ -59 (c 0.005, $CHCl_3$). Its molecular formula was established as $C_{12}H_{12}O_3$, derived from its HR-ESI-MS. The infrared radiation (IR) absorption spectra revealed characteristic bands at 1705, 1497, and 1361 cm^{-1} corresponding to aldehyde aromatic ring and *gem*-dimethyl functions. ^{13}C NMR spectrum confirmed the presence of aldehyde carbon signal resonating at δ_C 194.3 ppm, six signals in the aromatic region at δ_C 155.0, 131.3, 130.4, 128.0, 120.0, and 117.4 ppm, three further oxygenated carbons resonating at δ_C 79.3, 66.4, and 58.3 ppm, and two upfield signals at δ_C 26.8 and 19.4 ppm. ^{1}H NMR spectrum assigned the presence of two tertiary methyls at δ_H 1.26 and 0.95 ppm, two O-CH at δ_H 4.74 (d, *J* = 8.5 Hz) and 3.85 (d, *J* = 8.5 Hz) ppm, and three aromatic protons at 7.56 (br s), 7.70 (d, 8.5 Hz), and 7.22 (d, 8.5 Hz). Heteronuclear single-quantum correlation (HSQC)

Table 1: ^{1}H and ^{13}C Nuclear magnetic resonance spectral data for compounds 2 and 9

Position	2			9		
	δ_H	Mult. *J* in Hz	δ_C	δ_H	Mult. *J* in Hz	δ_C
1	-	-	-	-	-	149.5
2	-		79.3	3.98	dd, *J*=10.2, 4.3	74.4
3	3.85	d, *J*=8.5	66.4	1.72	m	42.8
				1.68	m	
4	4.74	d, *J*=8.5	58.3	5.11	dqd, *J*=11.1, 6.8, 3.4	68.2
5	7.56	br s	131.3	5.83	dd, *J*=9.4, 3.4	123.2
6	-	-	128.0	2.52	ddd, *J*=16.2, 9.4, 2.6	25.2
				2.11	ddd, *J*=16.2, 11.1, 3.4	
7	7.70	d, *J*=8.5	130.4	2.45	ddd, *J*=11.1, 10.2, 3.4, 2.6	48.3
8	7.22	d, *J*=8.5	117.4	4.31	ddd, *J*=12.8, 10.2, 3.4	82.4
9	-	-	155.0	2.32	ddd, *J*=12.8, 5.1, 3.4	36.0
				1.72	m	
10	-	-	120.0	2.81	dqd, *J*=11.1,6.8, 4.3	29.0
11	1.26	s	26.8	-	-	139.5
12	0.95	s	19.4	-	-	170.1
13	9.8	s	194.3	6.16	d, *J*=3.4	118.5
				5.44	d, *J*=3.4	
14				1.18	d, *J*=6.8	19.6
15				1.28	d, *J*=6.8	20.7
$OCCH_3$				-		171.7
CH_3CO				2.08	s	21.3
CH_3O				3.48	s	50.9

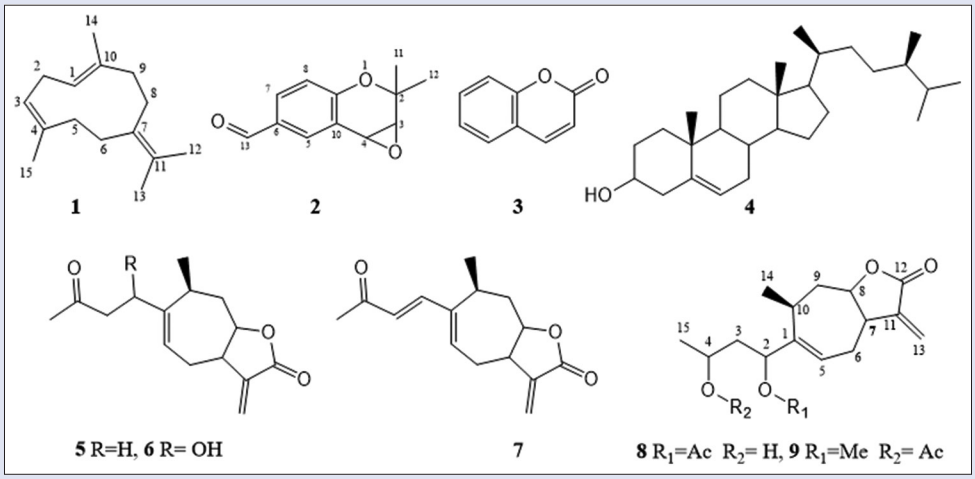

Figure 1: Structures of compounds isolated from *Xanthium strumarium*

spectrum assigned all protons to their carbon atoms. The multiplicity pattern with the calculated coupling constants (*J*) indicated the presence of 1,2,4-trisubstituted benzene ring. The molecular formula indicated that 2 has seven unsaturation sites, and the aldehyde function and the benzene ring accounted for five sites; therefore, 2 is a tricyclic compound. The presence of the oxirane ring was evidenced from the characteristic signals at δ_H/δ_C 3.85/66.4 and 4.74/58.3 ppm. The ^{1}H-^{1}H correlated spectroscopy (COSY) displayed two proton sequences, H-3/H-4 and H-8/H-7, which support the previous assumptions. Heteronuclear multiple-bond correlation (HMBC) spectrum indicated the presence of the pyran ring through correlations from H-4 to C-5,2,10, methyl protons (0.95 ppm) to C-3,2 and the other methyl carbon, together with the ^{13}C NMR signal at 155.0 ppm assigned to oxygenated aromatic carbon. Extensive interpretation of the HMBC results established the location of the aldehyde function through the correlations between aldehyde proton and C-6 together with that between H-5 and C-6 and the aldehyde carbon, as well as these two carbons with H-7. Compound 2 was previously identified as synthetic product.[14,15] It is a new natural product named 3,4-diepoxy-2,2-dimethyl-2H-1-benzopyran-6-carboxaldehyde.

Compound 9 was isolated as gummy material. Structure elucidation of compound 9 commenced after developing of a characteristic color of terpenes on spraying with *p*-anisaldehyde reagent. The IR absorption spectrum of 9 evidenced the presence of α,β–unsaturated-γ-lactone moiety (1767 cm^{-1}) which was validated by the UV absorption peak at 218 nm, in addition to acetyl group (1736 cm^{-1}). The molecular formula of 9 was established as $C_{18}H_{26}O_5$ from the HR-ESI-MS and requires six unsaturation sites. The ^{13}C NMR spectrum displayed 18 signals, two of which were ascribable to acetyl function (δ_C: 171.7 and 21.3) and one to methoxy carbon (δ_C: 50.9). The remaining 15 carbon atoms were categorized with the aid of distortionless enhancement by polarization transfer experiment into three CH-O functions (δ_C 82.4, 74.4, and 68.2), two C-CH(C)-C (δ_C 48.3 and 29.0), four CH_2 groups (δ_C 118.5, 42.8, 36.0, and 25.2), two methyl groups (δ_C 20.1 and 19.6),

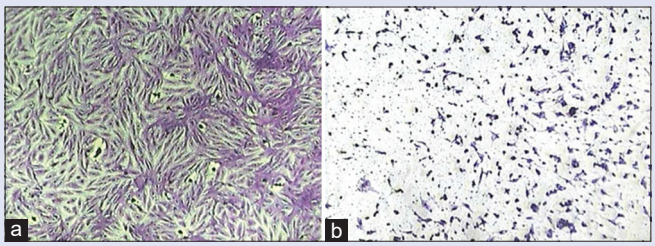

Figure 2: Morphological and cytological features of colorectal carcinoma cell line (HCT116) (a and b) treated with 1% dimethyl sulfoxide (vehicle control) (a), treated with compound 3 in a concentration of 100 μM (b) after 72 h of cell exposure to the investigated compound. (a) HCT116 grown as a control with dimethyl sulfoxide solvent, showing the characteristic monolayer of carcinoma cells, with the standard features of cellular atypia: nuclear and cytoplasmic pleomorphism, increased nucleus: cytoplasm ratio, highly irregularly-shaped cells (tadpole, caudate), irregular nuclear shapes, and hyperchromasia. (b) HCT116 with compound 3 addition shows a decreased number of colorectal carcinoma cells and necrotic cells in suspension

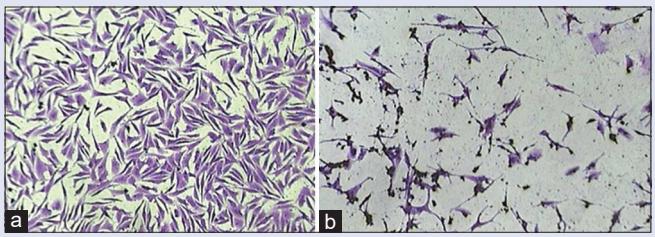

Figure 3: Morphological and cytological features of hepatocellular carcinoma cell line (HepG2) (a and b) treated with 1% dimethyl sulfoxide (vehicle control) (a), treated with compound 1 in a concentration of 100 μM (b) after 72 h of cell exposure to the investigated compound. (a) HepG2 grown as a control with dimethyl sulfoxide solvent, showing the characteristic monolayer of carcinoma cells, with the standard features of cellular atypia: nuclear and cytoplasmic pleomorphism, increased nucleus: cytoplasm ratio, highly irregularly-shaped cells (tadpole, caudate), irregular nuclear shapes, and hyperchromasia. (b) HepG2 with compound 1 addition shows a moderate decrease in the number of hepatocellular carcinoma cells and necrotic cells in suspension

one CH=(δ_C 123.2) along with three quaternary carbons including two all-carbon linked quaternary (δ_C 149.5 and 139.5), and one lactonized ketone group (δ_C 170.1) [Table 1]. In the ^{1}H NMR and HSQC spectra of **9**, in addition to two secondary methyls attached to saturated carbons (δ_H 1.28, d, J = 6.8 Hz and 1.18, d, J = 6.8 Hz), the δ_H value of the proton resonating at 4.31 (δ_H 4.31, ddd, J = 12.8, 5.1, and 4.3 Hz) suggested that the OH function at carbon (δ_C 82.4) was involved in a lactone ring. The presence of α,β-unsaturated-γ-lactone moiety was substantiated by the signals due to exocyclic methylene resonating at δ_H 6.16 and 5.44 with common J = 3.4 Hz. Based on the presence of two carbonyl functions, a trisubstituted double bond (δ_H 5.83, dd, J = 9.4 and 3.4 Hz), and a lactone ring, therefore, the compound 9 should be bicyclic. ^{1}H-^{1}H COSY spectrum established the presence of two proton sequences: H-2/H-3, H-3/H-4, and H-4/H-15, this was first, the second one was correlations observed as H-7/H-6, H-7/H-8, H-6/H-5, H-10/H-9, H-10/H-14, and H-9/H-8. HMBC spectrum showed correlations that established the presence of a seven-membered ring (H-5 at δ_H 5.83 with C-6,1,10 and H-9 at 4.31 with C-11,6,10), the location of the lactone ring (H-13 at 6.16 with C-12,11,7), and the location of the butyl side chain. The previous spectral data are most likely similar to xanthatin (7) metabolites frequently isolated from the plants of the genus *Xanthium*.[2-8] The positions of the methoxy and acetoxy functions were assigned to C-2 and C-4, respectively, based on the chemical shift values, correlations observed in HMBC, and comparison with data from the literature.[2-8] The name methoxy xanthanol was given to 9 [Figure 1].

Biology

The cytotoxic effect of the isolated compounds was evaluated against two cancer cell lines HCT116 and HepG2. Based on the cytotoxicity criteria of pure compounds (IC_{50} <4 μg/mL or <10 μM), the compounds were considered to be highly cytotoxic.[25,26] Compounds 3 and 4 showed significant cytotoxic activities against HCT116 cells with the mean IC_{50} values of 0.016 ± 0.005 and 0.246 ± 0.021 μM, respectively, while compounds 1,7-9 showed moderate cytotoxic activities against HCT116 cells with the mean IC_{50} values of 19.576 ± 1.16, 14.392 ± 1.32, 24.165 ± 2.81, and 25.834 ± 2.03 μM, respectively. Compounds 5 and 6 showed weak cytotoxic activities against HCT116 cells with the mean IC_{50} values of 75.495 ± 4.29 and 44.326 ± 3.70 μM, respectively, in comparison with doxorubicin (positive control) with a mean IC_{50} value of 0.41 ± 0.02 μM. Compounds 1, 3, and 7 showed moderate cytotoxic activities against HepG2 cells with the mean IC_{50} values of 10.231 ± 1.04, 14.319 ± 2.08, and 12.731 ± 1.93 μM, respectively, while compounds 5, 6, 8, and 9 showed weak cytotoxic activities against HepG2 cells with the mean IC_{50} values of 79.824 ± 5.09, 67.632 ± 5.76, 98.637 ± 7.13, and 42.839 ± 3.33 μM, respectively, in comparison with doxorubicin with a mean IC_{50} value of 0.74 ± 0.06 μM [Table 2 and Figures 2, 3].

Table 2: The cytotoxic activity (half maximal inhibitory concentration) of 9 compounds against the growth of HCT116 and HepG2 cells

Compound number	Colorectal carcinoma cell line (HCT116 ATCC® CCL-247™) (IC_{50} μM)	Hepatocellular carcinoma cell line (HepG2 ATCC® HB-8065™) (IC_{50} μM)
1	19.576±1.16	10.231±1.04
2	>100	>100
3	0.016±0.005	14.319±2.08
4	0.246±0.021	>100
5	75.495±4.29	79.824±5.09
6	44.326±3.70	67.632±5.76
7	14.392±1.32	12.731±1.93
8	24.165±2.81	98.637±7.13
9	25.834±2.03	42.839±3.33
Doxorubicin*	0.41±0.02	0.74±0.06

IC_{50}: Half-maximal inhibitory concentration

CONCLUSION

Nine natural compounds 1–9 were isolated from a medicinal plant, identified as *X. strumarium*. These compounds are derived from different metabolic pathways; interestingly, most of them showed an appreciated activity against HCT116 and HepG2 cancer cell lines.

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Nil.

Conflicts of interests

There are no conflicts of interest.

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